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Identification of yoghurt-spoiling yeasts with 18S rRNA-targeted oligonucleotide probes.

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ABSTRACT: 18S rRNA-targeted oligonucleotide probes were designed for rapid and reliable identification of yeasts involved in spoilage of dairy products. *Candida parapsilosis*, *Candida glabrata*, *Clavispora lusitaniae*, *Debaryomyces hansenii*, *Dekkera bruxellensis*, *Hanseniaspora uvarum*, *Pichia anomala*, *Pichia membranaefaciens*, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* and *Saccharomycopsis capsularis* were identified by dot blot hybridization assays using species-specific, digoxigenin-labeled probes which were deduced from comparative analysis of highly variable regions of 18S rRNA. The eucarya-specific probe EUK516 gave intense signals detected by epifluorescence microscopy when hybridized in situ to all yeast species tested. Whole cell hybridization experiments revealed that the 3'-end of the target molecule is a suitable site for fluorescently labeled species-specific nucleic acid probes which detect *S. cerevisiae*, *P. anomala*, *D. hansenii* and *D. bruxellensis* in situ. Other variable regions of the 18S rRNA tested for species-specific probes apparently were not accessible to in situ hybridization. *S. cerevisiae* and *P. anomala* were detected in yoghurt using the fluorescently labeled probes Sc1711 and Pan1710.

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Discrimination of **Brettanomyces/Dekkera** yeast isolates from wine by using various DNA finger-printing methods.

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JOURNAL: Food Microbiology (London) 16 (1):p3-14 Feb., 1999

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ABSTRACT: In the recent years **Brettanomyces/Dekkera** yeasts are posing an increasingly severe quality problem in the wine industry. The early and specific detection of these yeasts is therefore needed. Here, we describe the application of genetic techniques in addition to routinely performed physiological tests to identify yeasts isolated from Cabernet Sauvignon wines characterized by wine-makers panels as having 'betty' aromas. **Brettanomyces/Dekkera** reference strains from a type culture collection were included for comparison. A RAPD-PCR assay was developed for species- and strain-specific discrimination of these yeasts. These data were compared to the chromosomal patterns of uncleaved, Sfi 1-digested DNA, and to the physiological behavior of the yeasts. Karyotyping gave clear distinctions, but did not allow for relatedness studies due to the lack of pattern conservation among species and strains. Conversely, RAPD-PCR allowed for species discrimination within the genus **Brettanomyces** and strain discrimination within the species *D. bruxellensis*. All wine-isolated **Brettanomyces/Dekkera** yeasts belonged to the species *B. bruxellensis*. Populations derived from one single clone were found in the 1992 and 1994 vintage wines, but in contrast, the 1989 yeast population differed from these two vintages.- two different strains were found instead of a homogenous population. In conclusion, we show that RAPD-PCR can be successfully applied to discriminate **Brettanomyces/Dekkera** yeasts on the species and strain level representing an accurate alternative to conventional physiological tests.

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12046756 BIOSIS NO.: 199900327275

A new molecular method for simultaneous identification and enumeration of  
**Brettanomyces** in wine.

AUTHOR: Stender H(a); Perry-O'Keefe H(a); Hyldig-Nielsen J J(a); Broomer A  
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Rapid methods for detecting *Saccharomyces diastaticus*, a beer spoilage yeast, using the polymerase chain reaction.

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ABSTRACT: We have devised rapid methods for detecting *Saccharomyces diastaticus*, a beer spoilage microorganism, using polymerase chain reaction (PCR). We have designed primers to detect *S. diastaticus* by PCR, paying attention to sequential differences between the glucoamylase genes of *S. diastaticus* and *S. cerevisiae*. An examination of primer reactivity showed the forward primer, SD-5A, and the reverse primer, SD-5B, to react with *S. diastaticus* (seven strains tested); however, they did not react with other microorganisms, including the brewing yeast used by our company, two strains of *Saccharomyces* spp. 10 strains of *Brettanomyces* spp., four strains of wild yeast, four strains of fungus, and 23 strains of various bacteria. The DNA extracted enzymatically from cell numbers as low as 101 worked successfully as templates for the PCR method. Time required to extract DNA from cells and to detect *S. diastaticus* was only approx 5 hr. The combination of the rapid microbe detection system and PCR led to high accuracy of analysis. For a test of beer product, these combined procedures for the detection of *S. diastaticus* may be useful and, even from one cell which becomes one microcolony (one to two day old colony), a test can be completed in 30 hr.

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Identification of *Brettanomyces/Dekkera* yeasts from California wines.

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Author(s): Lopes MD (REPRINT) ; Soden A; Martens AL; Henschke PA; Langridge  
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Abstract: A **PCR**-based method has been developed that permits both intraspecies differentiation and species identification of yeast isolates, Oligonucleotide primers that are complementary to intron splice sites were used to produce **PCR** fingerprints that display polymorphisms between different species of indigenous wine yeasts, Although polymorphisms existed between isolates of the same species, the banding patterns shared several amplification products that allowed species identification. Importantly, the method was able to distinguish between species of the closely related *Saccharomyces sensu stricto* yeasts, In two cases where isolates could not be positively identified there was discrepancy between the phenetic and phylogenetic species concept; The method has applications in yeast ecological studies, enabling the rapid grouping of isolates with related genomes and the investigation of population dynamics of strains of the same species.